Woitach, Joseph

From: Woitach, Joseph
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J Embryol Exp Morphol. 1980 Dec;60:255-69.
Spielmann H, Jacob-Muller U, Beckord W.
Immunosurgical studies on inner cell mass development in rat and mouse blastocysts before and during implantation in vitro.

Anim Reprod Sci. 1998 Feb 27;50(1-2):1-9.
Stojkovic M, Buttner M, Zakhartchenko V, Brem G, Wolf E
A reliable procedure for differential staining of in vitro produced bovine blastocysts: comparison of tissue culture medium 199 and Menezo's B2 medium.

Thank you,

Joe

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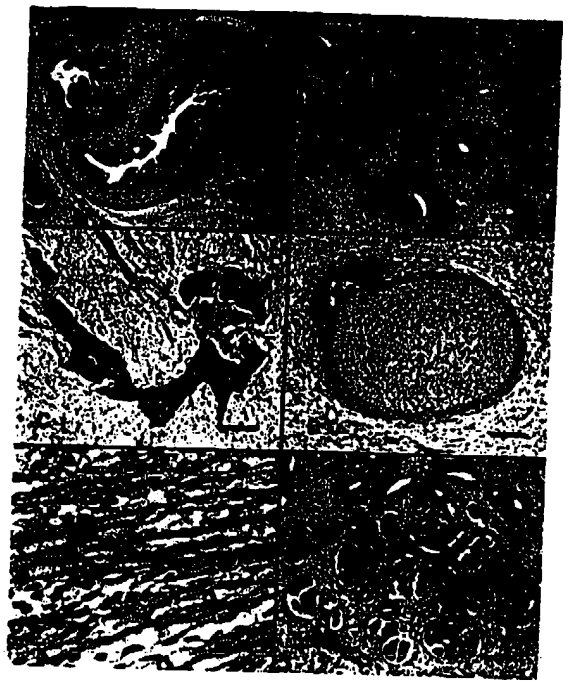

Fig. 4. Teratomas formed by the human ES cell lines in SCID-beige mice. Human ES cells after 4 to 5 months of culture (passages 14 to 16) from about 50% confluent six-well plates were injected into the rear leg muscles of 4-week-old male SCID-beige mice (two or more mice per cell line). Seven to eight weeks after injection, the resulting teratomas were examined histologically. (A) Gutlike structures. Cell line H9. Scale bar, 400 μm. (B) Rosettes of neural epithelium. Cell line H14. Scale bar, 200 μm. (C) Bone. Cell line H14. Scale bar, 100 μm. (D) Cartilage. Cell line H9. Scale bar, 100 μm. (E) Striated muscle. Cell line H13. Scale bar, 25 μm. (F) Tubules interspersed with structures resembling fetal glomeruli. Cell line H9. Scale bar, 100 μm.

transplantation therapies. Many diseases, such as Parkinson's disease and juvenile-onset diabetes mellitus, result from the death or dysfunction of just one or a few cell types. The replacement of those cells could offer lifelong treatment. Strategies to prevent immune rejection of the transplanted cells need to be developed but could include banking ES cells with defined major histocompatibility complex backgrounds or genetically manipulating ES cells to reduce or actively combat immune rejection. Because of the similarities to humans and human ES cells, rhesus monkeys and rhesus ES cells provide an accurate model for developing strategies to prevent immune rejection of transplanted cells and for demonstrating the safety and efficacy of ES cell–based therapies. Substantial advances in basic developmental biology are required to direct ES cells efficiently to lineages of human clinical importance. However, progress has already been made in the in vitro differentiation of mouse ES cells to neurons, hematopoietic cells, and cardiac muscle (*22–24*). Progress in basic developmental biology is now extremely rapid; human ES cells will link this progress even more closely to the prevention and treatment of human disease.

References and Notes

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6. Thirty-six fresh or frozen-thawed donated human embryos produced by IVF were cultured to the blastocyst stage in G1.2 and G2.2 medium (*25*). Fourteen of the 20 blastocysts that developed were selected for ES cell isolation, as described for rhesus monkey ES cells (*5*). The inner cell masses were isolated by immunosurgery (*26*), with a rabbit antiserum to BeWO cells, and plated on irradiated (35 grays gamma irradiation) mouse embryonic fibroblasts. Culture medium consisted of 80% Dulbecco's modified Eagle's medium (no pyruvate, high glucose formulation; Gibco-BRL) supplemented with 20% fetal bovine serum (Hyclone), 1 mM glutamine, 0.1 mM β-mercaptoethanol (Sigma), and 1% nonessential amino acid stock (Gibco-BRL). After 9 to 15 days, inner cell mass–derived outgrowths were dissociated into clumps either by exposure to Ca^{2+}/Mg^{2+}-free phosphate-buffered saline with 1 mM EDTA (cell line H1), by exposure to dispase (10 mg/ml; Sigma; cell line H7), or by mechanical dissociation with a micropipette (cell lines H9, H13, and H14) and replated on irradiated mouse embryonic fibroblasts in fresh medium. Individual colonies with a uniform undifferentiated morphology were individually selected by micropipette, mechanically dissociated into clumps, and replated. Once established and expanded, cultures were passaged by exposure to type IV collagenase (1 mg/ml; Gibco-BRL) or by selection of individual colonies by micropipette. Clump sizes of about 50 to 100 cells were optimal. Cell lines were initially karyotyped at passages 2 to 7.
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16. Alkaline phosphatase was detected with Vector Blue substrate (Vector Labs). SSEA-1, SSEA-3, SSEA-4, TRA-1-60, and TRA-1-81 were detected by immunocytochemistry with specific primary monoclonal antibodies and localized with a biotinylated secondary antibody and then an avidin or biotinylated horseradish peroxidase complex (Vectastain ABC system; Vector Laboratories) as previously described (*5*). The ES cell lines were at passages 8 to 12 at the time markers were analyzed.
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20. hCG and α-fetoprotein were measured by specific radioimmunoassay (double AB hCG and AFP-TC kits; Diagnostic Products, Los Angeles, CA). hCG assays used the World Health Organization Third International Standard 75/537. H9 cells were allowed to grow to confluence (day 0) on plates of irradiated mouse embryonic fibroblasts. Medium was replaced daily. After 2 weeks of differentiation, medium in triplicate wells conditioned for 24 hours was assayed for hCG and α-fetoprotein. No hCG or α-fetoprotein was detected in unconditioned medium.
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27. We thank the personnel of the IVF clinics at the University of Wisconsin School of Medicine and at the Rambam Medical Center for the initial culture and cryopreservation of the embryos used in this study; D. Gardner and M. Lane for the G1.2 and G2.2 media; P. Andrews for the NTERA2 cl.D1 cells and the antibodies used to examine cell surface markers; C. Harris for karyotype analysis; and Geron Corporation for the 293 and MDA cell pellets and for assistance with the telomerase TRAP assay. Supported by the University of Wisconsin (UIR grant 2060) and Geron Corporation (grant 133-BU18).

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1: Proc Natl Acad Sci U S A. 1975 Dec;72(12):5099-102.

Immunosurgery of mouse blastocyst.

Solter D, Knowles BB.

Mouse blastocysts with and without zonae pellucidae are susceptible to complement-dependent antibody cytotoxicity. Exposure of blastocysts to rabbit anti-mouse serum together with complement results in the death of all cells; however, when blastocysts are exposed to antiserum alone and then transferred to guinea pig complement, only the trophoblastic cells are killed. These results suggest that the mouse blastocyst is not permeable for certain antibodies. The inner cell masses can easily be separated from the remnants of trophoblastic cells and are then able to grow and differentiate in vitro. This method of immunosurgery can be used to obtain large quantities of pure inner cell masses in a relatively short period of time.

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